



US005547861A

**United States Patent** [19][11] **Patent Number:** **5,547,861****Nadeau et al.**[45] **Date of Patent:** **Aug. 20, 1996**[54] **DETECTION OF NUCLEIC ACID AMPLIFICATION**[75] **Inventors:** James G. Nadeau; George T. Walker, both of Chapel Hill, N.C.[73] **Assignee:** Becton, Dickinson and Company, Franklin Lakes, N.J.[21] **Appl. No.:** 229,281[22] **Filed:** Apr. 18, 1994[51] **Int. Cl.<sup>6</sup>** ..... C12P 19/34; C12Q 1/70; C12Q 1/68; C07H 21/04[52] **U.S. Cl.** ..... 435/91.2; 435/5; 435/6; 536/24.3[58] **Field of Search** ..... 435/91.2, 6, 5; 536/24.3-33[56] **References Cited****U.S. PATENT DOCUMENTS**

5,126,239	6/1992	Livak et al.	435/6
5,210,015	5/1993	Gelfand et al.	
5,348,853	9/1994	Wang et al.	435/6

**FOREIGN PATENT DOCUMENTS**

0420260	4/1991	European Pat. Off.
WO90/06374	6/1990	WIPO
WO92/01812	2/1992	WIPO
WO92/02638	2/1992	WIPO
WO92/11390	7/1992	WIPO

**OTHER PUBLICATIONS**

Walker et al., Strand displacement amplification—an isothermal in vitro DNA amplification technique, NAR 20: 1691-1696, 1992\*.

WO9201812—Uhlen et al. Competitive PCR for quantitations of DNA, pp. 1-19, pub. Feb. 6, 1992\*.

G. T. Walker, et al. "Isothermal in vitro amplification of DNA by a restriction enzyme/DNA polymerase system" *Proc. Natl. Acad. Sci. USA* 89, 392-396 (1992).C. P. H. Vary "Triple-Helical Capture Assay for Quantification of Polymerase Chain Reaction Products" *Clin. Chem.* 38, 687-694 (1992).J. Wahlberg, et al. "General colorimetric method for DNA diagnostics allowing direct solid-phase genomic sequencing of the positive samples" *Proc. Natl. Acad. Sci. USA* 87, 6569-6573 (1990).D. J. Kemp, et al. "Colorimetric detection of specific DNA segments amplified by polymerase chain reactions" *Proc. Natl. Acad. Sci. USA* 86, 2423-2427 (1989).F. F. Chehab, et al. "Detection of specific DNA sequences by fluorescence amplification: A color complementation assay" *Proc. Natl. Acad. Sci. USA* 86, 9178-9182 (1989).A. C. Syvanen, et al. "Quantification of polymerase chain reaction products by affinity-based hybrid collection" *Nucl. Acids Res.* 16, 11327-11338 (1988).A. Chan, et al. "Quantification of Polymerase Chain Reaction Products in Agarose Gels with a Fluorescent Europium Chelate as Label and Time-Resolved Fluorescence Spectroscopy" *Anal. Chem.* 65, 158-163 (1993).C. R. Newton, et al. "The production of PCR products with 5' single-stranded tails using primers that incorporate novel phosphoramidite intermediates" *Nucl. Acids. Res.* 21, 1155-1162 (1993).

(List continued on next page.)

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Methods for detecting, immobilizing or localizing primer extension products of a Strand Displacement Amplification reaction which are coupled to, and an indication of, amplification of the target sequence. The primer extension products are secondary, target-specific DNA products generated concurrently with SDA of the target sequence and can therefore be used to detect and/or measure target sequence amplification in real-time. In general, the secondary amplification products are not amplifiable and remain inert in the SDA reaction after they are formed without interfering with amplification of the target sequence. The secondary amplification products may be designed or modified to contain special features to facilitate their detection, immobilization or localization.

**20 Claims, 3 Drawing Sheets**